EVALUATION OF SEALING ABILITY AND CYTOTOXICITY OF CASTOR OIL POLYMER CEMENT AS ROOT END FILLING MATERIAL –AN IN VITRO STUDY

A Dissertation submitted

in partial fulfillment of the requirements

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certificate

This is to certify that **Dr.P.BASKAR,** post graduate student (2008 - 2011) in the Department of Conservative Dentistry and Endodontics,has done this dissertation titled "**EVALUATION OF APICAL SEAL AND AMIBACTERIAL EFFICACY AGAINST E.FAECALISDY ”** under our direct guidance and supervision in partial fulfillment of the regulations laid down by **The Tamil Nadu Dr. M.G.R. Medical University, Guindy, Chennai – 32** for **M.D.S.**inConservative Dentistry and Endodontics (Branch IV) Degree Examination.

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DECLARATION

TITLE OF DISSERTATION	**EVALUATION OF SEALING ABILITY AND CYTOTOXICITY OF CASTOR OIL POLYMER CEMENT AS ROOT END FILLING MATERIAL –AN IN VITRO STUDY**
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Introduction

Periradicular surgery has continued to evolve into a precise, biologically based adjunct to non surgical root canal therapy. The underlying etiology of the disease process and the objectives of treatment are the same: prevention or elimination of apical periodontitis. Periradicular surgery is indicated when nonsurgical retreatment is impractical or unlikely to improve on the previous result.

Etiologic factors typically can be categorized as intraradicular or extraradicular bacteria,intraradicular or extraradicular chemical substances or extraradicular physical factors[48,59, 60] .Most cases involve some form of bacterial participation –within apical ramification .The only definitive means of eradicating such an irritant is physical removal through root end resection . The rationale for ***root-end resection*** in such cases is to establish access to and remove the diseased tissues.

Approximately 75% of teeth have canal aberrations (e.g. Accessory or lateral canals) in the apical 3mm of the tooth [17, 57] .An apical resection of approximately 3mm should include most accessory and lateral canal , thus eliminates most residual microorganisms and irritants when roots with more than one main canal are resected. However tissue may be present in isthumus , and the preparation should be modified to include the isthumus area.

The ***root end cavity preparation*** is a crucial step in the establishment of an apical seal. The goal is to make a cavity in the resected root end that is dimensionally sufficient for placement of a root end filling material and at the same time avoid unnecessary damage to the root end structures. With the advent of ultrasonic tips designed specifically for

this purpose, root end preparations now are most often performed with the ultrasonic techniques[14]

The ***ideal root end filling material*** seals the contents of the root canal system within the canal, preventing egress of any bacteria, bacterial byproducts, or toxic material into the surrounding periradicular tissues. The material should be non resorbable, biocompatible, and dimensionally stable over time. It should be able to induce regeneration of PDL complex, specifically cementogeneis over the root end filling itself. Finally handling properties and working time should be such that the surgeon can place root end filling with sufficient ease.

Many materials are being used as root end filling including Guttapercha,Polycarboxylate Cement,Silvercones, Amalgam, Cavit ,Zinc Phosphate Cement ,Gold Foil, and Titanium Screws,Glass ionomer Cement , Diaket,Composite Resins ,Resin Glass Ionomer Hybrids and MTA .

All these root end filling materials have specific ***advantages and disadvantages*** .However from the biologic perspective of regeneration of the periradicular tissues, MTA followed by Retroplast , appears to have a clear advantage over the other available materials. Retroplast and other composite resin based filing materials require meticulous homeostasis and a dry surgical field for optimal results .The most commonly cited disadvantage of MTA is its handling properties. Even when properly prepared, MTA is more difficult to place in the root end cavity than most other materials.

When a non adhesive material is used for apical sealing, a microscopic space always exists between the restoration and the tooth which leads to microleakage [69]. ***Micro-***

leakage is defined as flow of oral fluid and bacteria into the microscopic gap between a prepared tooth surface and a restorative material[38]

A relatively ***new material, Castor Oil Polymer cement (COPC)***, has demonstrated a good potential as root-end filling material. This material has been widely used in medicine for prostheses to replace bones because it is biocompatible, nontoxic and easy to handle. The COPC is a common tropical climate plant (*Riccinus communis*) extract with excellent potential for oil production, promoting a large scale supply of polyol and fatty acid prepolymers[50]. This biopolymer has high interaction capacity with human cells. The chemical composition of this material presents a chain of fatty acids whose molecular structures are also present in lipid of human body[69].Therefore, the cells do not recognize the COPC as a foreign body.

Before starting a cell culture transport test or in vivo experiments, it is essential to carry out preliminary in vitro tests to screen and characterize the potential harmful effects of a material to the tissues. The concentration- dependent cytotoxicity of the substances can be determined by ***cytotoxicity tests***. The cytotoxicity of material is done by various in-vitro methods. It is critical to select the appropriate cell types for cytotoxicity

Aim and objectives :

The aim of this present study were :

a) To compare the micro leakage of castor oil polymer cement with IRM and MTA using dye penetration method

b) To evaluate the cytotoxicity of castor oil polymer cement by MTT assay using NIH 3T3 mouse fibroblast

Review of literature

The goal of endodontic therapy is to obtain impermeable seal apically and coronally. Properly performed with suitable materials, retrograde fillings is a clinically valuable procedure promoting the prognosis of apical surgery, particularly when orthograde obturation is not performed in conjunction with surgery. Despite its clinical value, retrograde filling should be considered second alternative to retrograde endodontic treatment.

ROOT END FILLING MATERIALS

Bramwell JD et al[12](1986) evaluated the Sealing ability of four retro- filling techniques , in which there were four groups -apicoectomy alone, apicoectomy with an amalgam retro-filling, apicoectomy with heat sealed gutta percha and apicoectomy with cold burnished gutta percha were compared. There was no significant difference among the four experimental groups. The results indicated the retro filling procedures that use amalgam or gutta percha produce apical seal of comparable quality.

Torabinejad M et al[68] (1994) evaluated the dye leakage of four root end filling materials .Amalgam, super EBA, IRM and mineral trioxide aggregate were evaluated for their ability to prevent apical dye leakage in vitro with or without blood present in the root end preparation. The results showed that presence or absence of blood had no significant effect on the amount of dye leakage. However, there was a significant leakage difference between the

root end filling materials where Mineral trioxide aggregate leaked significantly less than the other materials.

Giheany PA et al [28] (1994) evaluated the apical leakage associated with various depths of retrograde filings placed in root apices with a hydraulic conductance apparatus. The results indicated that the increasing depth of retrograde filling significantly decreased apical leakage and also there was a significant increase as the amount of bevel increased.

Higa RK et al [32](1994) performed dye leakage study between super EBA, IRM and amalgam where super EBA and IRM showed significantly less dye leakage than amalgam. No significant difference existed between super EBA and IRM. It was observed that there was no significance of storage time on the amount of dye leakage.

Kettering JD et al[41](1995) examined Mutagenic potential of IRM, super EBA and MTA using Ames test. The results indicated that MTA, IRM and super EBA do not appear to be mutagenic.

Torabinejad M et al [69] (1995) compared various physical and chemical properties like chemical composition: pH, radiopacity, setting time, compressive strength and solubility of MTA with those of amalgam, super EBA and intermediate restorative material (IRM). The results showed that the main molecules present in MTA were calcium and phosphorus ions, the initial pH was 10.2 which increased to 12.5 in 3 hours after mixing,

it was more radiopaque than super-EBA and IRM. Setting time of amalgam was 4 minutes and MTA had the longest setting time of 2 hours 45 minutes. MTA had the lowest compressive strength that had increased after 21 days. In terms of solubility only IRM showed solubility under the conditions of this study

Bates CF et al [7] (1996) in their study found that MTA seals more effectively to the root end when compared with amalgam and Super EBA, thus it was determined to be a better material in preventing microleakage

Min MM et al [48] (1997) used Confocal microscopy to investigate structural alterations in resected roots that had root end preparations made with a conventional microhead handpiece and ultrasonics at two intensity levels. Results of the histological data indicated that root ends prepared by ultrasonics had a significantly greater number of fractures than roots prepared with conventional microhead handpiece

Lin CP et al [44] (1998) evaluated the quality of root end preparation techniques involving specially designed ultrasonic retrotip and with those prepared in a traditional manner with a micro handpiece bur. The results showed that the ultrasonic root end preparation produced more conservative cavities with less of perforations when compared with conventional micro handpiece bur preparations.

Fischer EJ et al [23] (1998) compared bacterial leakage of Mineral Trioxide Aggregate with zinc-free amalgam, Intermediate Restorative Material, and super EBA as a root

end filling material. The results showed that most of the samples with zinc-free amalgam leaked bacteria in 10-63 days, IRM had begun leaking in 28-91 days, Super EBA began leaking in 42-101 days whereas MTA did not begin leaking until day 49. At the end of the study, four of the MTA samples had not exhibited any leakage. The results indicated MTA to be the most effective root end filling material against penetration by Serratia marscescens.

Koh ET et al [42] (1998) in their study investigated the cytomorphology of osteoblasts in presence of MTA and examined cytokine production comparing with IRM. The results of SEM revealed healthy cells in contact with MTA at 1 and 3 days. In contrast cells in presence of IRM appeared rounded. ELISA assay revealed raised levels of interleukins at all periods whereas cell amounts with IRM were undetectable. Thus MTA offered a biologically active substrate for bone cells and stimulated IL production.

Sauveur G et al [58] (1998) carried out Photoelastimetric analysis, of stress induced by root end resection at angle of 45° was compared to when resection angle was kept perpendicular to long axis of the roots. It was found that root end resection perpendicular to the long axis offered better distribution of stresses exerted on the apical region than a 45° resection.

Gagliani M et al [26] (1998) in their study evaluated the influence of apical root end resection angle and the cavity made by ultrasonic retrotip on the apical seal. Apical leakage was determined using fuchsin dye. The results showed apical cavity of 3mm or more along the vertical axis i.e. 90° angle could produce a safe and effective seal.

Adamo HL et al [1](1999) compared four root end filling materials MTA,super EBA, composite and amalgam for resistance to bacterial microleakage. The rate of microleakage tested at intervals of 4, 8 and 12 weeks showed no significant difference statistically.

Torabinejad M et al [67] (1999) described in the clinical procedure of application of MTA as pulp capping with reversible pulpitis, apexification, non-surgical repair of root perforations and as well as its use as a root end filling material .

Fogel HM et al [24] (2001) evaluated Microleakage of amalgam, Intermediate Restorative Material (IRM), a dentin bonding resin, Super-EBA and mineral trioxide aggregate (MTA) using a fluid filtration system. It was found that amalgam demonstrated more microleakage than Super-EBA, dentin-bonding resin, or MTA. There was no significant difference between amalgam and IRM.

Economides N et al [21] (2003) evaluated the short-term response of periradicular tissues to MTA when used as a root-end filling material in ideal tissue conditions . It was proved that MTA was biocompatible material that stimulated periradicular tissue repair at the root-end situation

Gondim E et al [29] (2003) evaluated marginal adaptation using surface topography of root apices following ultrasonic root-end preparation and again after root-end fillings submitted to three different finishing techniques. It was concluded that the marginal

adaptation of MTA was good with or without finishing procedures. Applying a finishing bur over the condensed and set IRM and Super EBA created better marginal adaptation.

Perez AL et al [53] (2003) A hypothesis that MG-63 osteosarcoma cells and primary osteoblasts react differently to ProRoot MTA and White MTA was confirmed by

a) Investigating the attachment of primary osteoblasts and MG-63 osteosarcoma cells to ProRoot MTA with White MTA.

b) Comparing the osteogenic behavior of both cell lines in contact with these endodontic materials.

It was concluded that MG- 63 cells do not behave osteogenically by forming mineralized nodules and primary osteoblasts were more sensitive than MG-63 osteosarcoma cells to White MTA in cell culture. Primary osteoblasts were more appropriate than MG- 63 cells for testing endodontic materials in cell culture.

Pistorius A et al [54] (2003) evaluated the influence of mineral trioxide aggregate, amalgam and chemically inert titanium alloy on specific cellular responses of gingival fibroblasts. MTA demonstrated cellular responses similar to those of titanium, while amalgam showed an irritation rate higher than that of MTA and titanium.

Sousa CJ et al [63] (2004) evaluated the biological properties of zinc oxide eugenol, mineral trioxide aggregate and Z-100 light cured composite resin. It was concluded that MTA and Z-100 composite were biocompatible at 4 and 12 weeks whereas ZOE cement was highly toxic during the 4th week experimental period but by 12th week it showed biocompatible characteristics.

Santos AD et al [57](2005) evaluated the release of calcium ions, pH and conductivity of new experimental dental cement (EC) when compared with those of MTA-Angelus. The experimental cement released calcium and increased the pH of the storage solutions in a similar manner to MTA-Angelus. However experimental cement showed significantly higher calcium release than commercial MTA-Angelus after 24 hours.

Mário et al [45] (2005) analyzed the effect of different dyes on the evaluation of the apical sealing ability of Mineral Trioxide Aggregate root-end fillings. It was concluded that the evaluation of the sealing ability of MTA is influenced by the dye used, since this material presented better sealing ability when evaluated with Methylene Blue, but was similar to ZOE when evaluated with rhodamine B.

Beatriz Farias Vogt et al [8] (2006) evaluated the penetration of three dyes in MTA root-end fillings. Within the limitations of the methodology applied, it was possible to conclude that the dyes tested presented different degrees of penetration into apical dentin. The lowest leakage results were observed for silver nitrate and the highest penetration, for rhodamine. Methylene presented intermediate results.

CASTOR OIL POLY URETHANE

José Carlos Garcia de Mendonça et al [38] (200)carried out morphological study comparing castor oil polyurethane and autogenous bone graft to repair bone defect in zygomatic bone of rabbits. Fibrous connective tissue encapsulated the polyurethane, but no inflammation or giant cell reaction was observed. The castor oil polyurethane was biocompatible and did not cause inflammation. It may be considered an alternative to fill bone defects.

Martin et al [47] **(2009)** evaluated the sealing ability of castor oil polymer (COP), mineral trioxide aggregate (MTA) and glass ionomer cement (GIC) as root-end filling materials. The results of this study indicate that the COP presented efficient sealing ability when used as a root-end filling material showing results significantly better than MTA and GIC

Adriana-Socorro-Ferreira Monteiro et al [2] (2010) analyzed the biocompatibility and degradation process of biomembranes. The morphological changes in subcutaneous implantations were assessed after 7, 14, 21, 28 and 70 days. Both polyurethane polymer (AUG) obtained from vegetable oil (Ricinus communis)and polytetrafluoroethylene membrane (PTFE). Histological analysis showed moderate inflammatory infiltrate, which was predominantly polymorphonuclear. There was also a presence of edema, which was gradually replaced by granulation tissue, culminating in a fibrous capsule. The researched material is biocompatible and the degradation process is extremely slow or may not even occur at all.

CYTOTOXICITY

Bean et al [9](1995), compared the MTT Colorimetric method and ^{51}Cr release assay for cytotoxicity determination of dental biomaterials and found that MTT assay showed more sensitivity and has less potential of binding interface than ^{51}Cr release assay.

Ratanasathien et al[56] (1995) observed the interactive effects synergism, additive effects , antagonism of four important resin components in 3T3 mouse fibroblast cultures and demonstrated the ranking of toxicity of dentin bonding components as follows :BisGMA>UDMA>TEGDMA>HEMA after 24 hrs &72 hrs exposures .

Douglas R.Rakich et al [20] (1998) determined the concentrations of the components of dentin bonding agents that suppress the mitochondrial activity of human macrophages in viro using MTT assay and found that the effect was time dependent

V.Geurtsen et al[27] (1998) determined the cytotoxic effects of 35 monomers in commercial dental resin composites using primary 3T3 fibroblast and three human primary fibroblast cultures .Their results indicated that primary human periodontal ligament and pulp fibrolast were found to be more sensitive than 3T3 fibroblast and gingival fibroblasts to alteration from most tested substances .

J.C.Wataha et al [77] (1999) tested the cytotoxicity of resin containing restorative material after aging in artificial saliva using 3T3 fibroblast in culture. They

assessed the succinate dehydrogenase activity using the MTT assay . Their results showed that all those materials continue to release sufficient components to cause lethal effects or alter cellular function invitro even after 2 weeks of aging in artificial saliva

Esther Navarro-Escobar et al [22] (2010) evaluate the cytotoxicity of 15% citric acid, 5% phosphoric acid and 2.5% NaOCl on cultured 3T3 fibroblasts using MTT colorimetric assay. The irrigating solution with the highest percentage of cell viability was 2.5% NaOCl at both 0.1% and 0.5% dilutions. A very low percentage of cell viability was obtained with 15% citric acid and 5% phosphoric acid at 0.5% dilution.

Materials and methods

Armamentarium used (fig no.1,2,6,7,8,9,10,11,12,13&15)

- Airotor hand piece(NSK High speed handpeice)
- Straight hand piece(NSK slow speed handpiece)
- Contrangle hand piece(NSK slow handpiece)
- Wheel diamond disc
- Burs and diamond points
- Cross cut fissure , tungsten carbide bur (no.55)
- K files (15-80)
- Barbed broaches
- Finger spreaders
- Vice
- Stereomicroscope
- Incubator
- Laminar flow cabinet
- UV spectrophotometer (ELCO CL3)
- Micropipettes
- 24 well plates
- Three neck roound flask and stirrer

Materials used (fig no.2,3,4,5&14)

- Intermediate Restorative material(IRM)(caulk,Dentsply)
- Mineral tri oxide aggregate(MTA)(Angelus ,Londrina ,PR Brazil)
- Castor Oil Polymer Cement (COPC)
- Glass Ionomer cement (GC FUJI PLUS)
- Normal saline
- 5.25% sodium hypochlorite
- 17%EDTA
- Gutta-percha
- Absorbent points
- Zinc oxide eugenol
- 2% Rhodamine dye
- Polycaprolactone Diol (polyol)
- Tetra hydro furan (THF)
- 1 -6 , di- isocyanate hexane
- Dibutyltin dilaurate(DBTL)
- Nail varnish
- Human maxillary central incisors
- Dulbecco's Modified Eagle's Medium
- Phosphate buffered saline

Fig No. 1

Fig no.2

Fig no. 3

Fig no : 4

Fig no.5 Polycaprolactone Diol (polyol),1 -6 , di- isocyanate hexane & Dibutyltin dilaurate(DBTL)

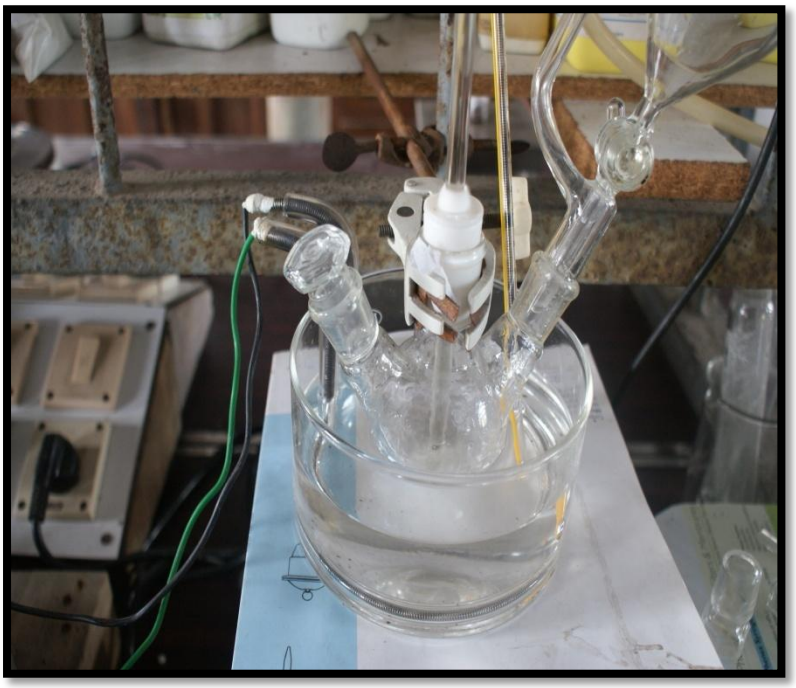


Fig no.6 Three neck flask with stirrer

Fig no.7 Optical stereomicroscope

Fig no. 8 Vice for holding teeth

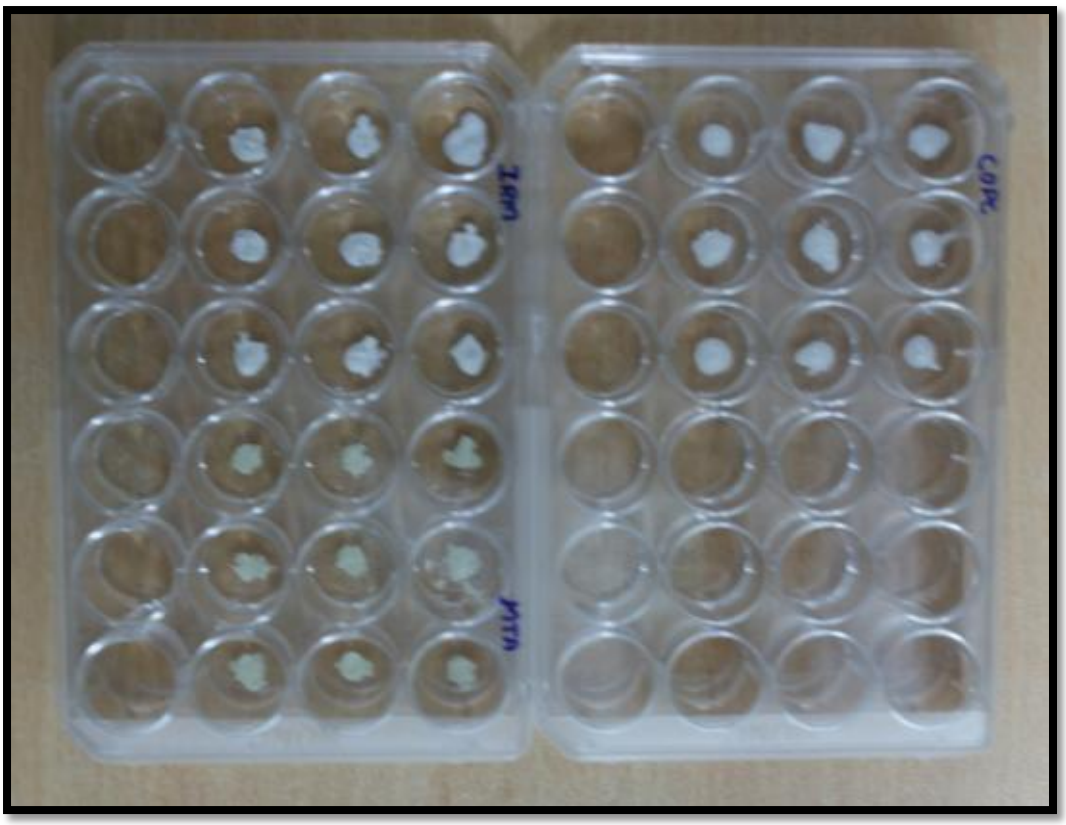


Fig no .9 24 well plates

Fig no.10 Incubator

Fig no 11 Laminar flow

Fig no. 12 Micropipettes

Fig no : 13 Phase contrast microscope

Fig no. 14 DMEM and PBS

Fig no .15 UV Spectrophotometer

Castor oil polymer cement

Castor oil also known as Riccinus oil is extracted from the castor oil plant , a typical bush from tropical climates . Vegetable oils are triglycerides, basically composed of fatty acids. Castor oil is mainly composed by the molecule of ricinoleic acid, with a chemical structure similar to the essential human fatty acids like the linoleic acid and the alpha hidroxy nervonic acid, which is found in the human nervous system.

Castor oil polymer cement is composed of a pre polymer derived from isocyanate, castor oil and calcium carbonate. (fig no.18)

The chemical reaction between two or more isocyanates and castor oil forms more than one radical of urethane. This is also called as polyurethane. This reaction was proposed by Wurtz in 1849, and first castor oil urethane was synthesized before 1950, to be used in paints and varnishes.

R1 -N=C=O + R2- O- H ⟶ R1- NH- C = O- O -R2

Isocyanate hydroxila urethane

Preparation of pre polymer

The polymerizations were carried out under nitrogen atmosphere in a 250 mL three neck flask equipped with a mechanical stirrer. Polycaprolactone Diol (polyol) is stirred under 70^0C under the inert nitrogen medium. Polyol was dissolved in solvent Tetra hydro furan (THF) and then heated to 70^0 C for 30 minutes .1,6 di- isocyanate hexane in THF is slowly added in order to yield the isocyanate (NCO) terminated pre polymer .After 30 min,001% of dibutylin dilaurate (DBTL) in THF (the catalyst) was added. The reaction temperature was raised to 70^0 C and maintained for at least two hours until NCO groups were reacted.(fig no.16)

Fig no:16 Preparation of pre polymer

Manipulation:

The prepared pre polymer is stored in sealed container to avoid the cross link between the NCO group, when exposed to moisture. The pre polymer and the castor oil are mixed in 1:1.5 ratios and the calcium carbonate is added 50 % of combined weight of pre polymer and castor oil. Calcium carbonate is added as filler.(fig no 19)

The castor oil polymer cement is mixed in dry glass slab and the components are mixed to get a thin luting consistency. The initial setting time is 20 minutes and final setting time is about 4 hours.

Fig no.17

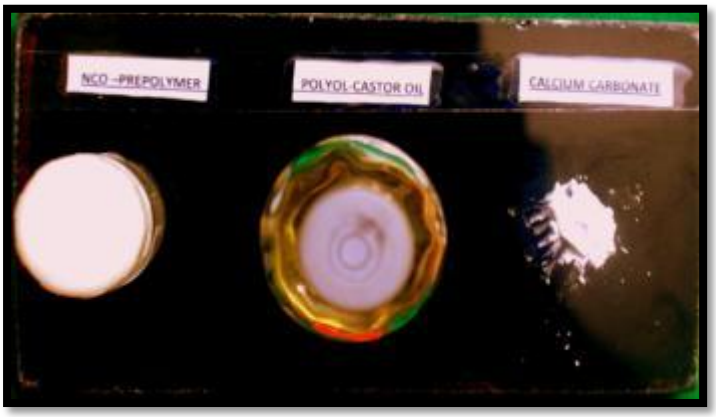


Fig no.18

Fig no. 19 (mixing of COPC)

Properties:

- Adhesive state observed in 3- 5 minutes
- "Malleable state" 5- 10 minutes**,** for complete solidification it takes around 20 minutes, and material resembles hardened plastic.
- Reaction is additional polymerization, no catalyst required, no free radicals. Does not liberate residual monomers
- slightly exothermic (around 40 ^{0}C)

Sample Processing

Sixty freshly extracted human maxillary central incisors with completely formed apices and straight canals were selected .Calcified canals,tortuous canals and teeth with root caries are excluded(figno.20) The teeth were cleaned and sectioned at CEJ using a diamond disc standardizing the root length to approximately 16mm . The pulp tissue was extirpated with a barbed broach 15 No.K file was used to confirm patency .The working length is determined with the help of radiographs. Canals enlarged up to No.50 by a standardized step back technique and irrigated with 5 ml of 5.25% NaOCl solution after each instrument was changed. Then the root canals were filled with 17% EDTA for 3 min .All the teeth were obturated with gutta-percha by lateral condensation technique using zinc oxide eugenol as sealer. (fig no.21). The cervical access was sealed with glass ionomer cement. The apical 1 mm of each root is resected perpendicularly to the long axis using diamond disc(fig no.22). A root end cavity 3 mm was prepared using straight fissure bur using slow speed handpiece .
.(fig no.23)

The teeth were randomly divided into three experimental groups and one control group (n=15)

Group A control (without root end filling material.)

Group B IRM (Intermediate restorative material)

Group C MTA(Mineral trioxide aggregate)

Group D Castor Oil Polymer Cement (COPC)

The materials were manipulated according to the manufacturer's instructions and the above mentioned method for COPC .The root end cavities were filled.(fig no.24)

Specimens were stored in moist cotton and then coated with nail varnish except the margins of the root end cavity and then allowed to dry(fig no.25). The specimens were suspended in 2% Rhodamine B dye for 24 hrs. Following this the roots were rinsed for 1 hour under tap water. (fig no.26)

The teeth were split longitudinally with a diamond disc using a water coolant(fig no.27) .The depth of dye penetration was examined under stereomicroscope(2X magnification) and micro leakage associated with different root end filling materials were evaluated in millimeters.

Fig no.:20 Specimens of maxillary incisors

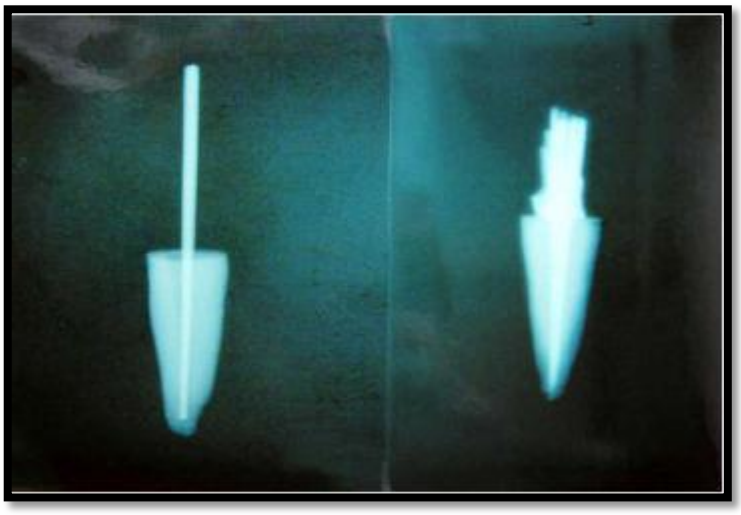


Fig no.:21 Radiographic picture of obturation

Fig no.:22 Root end resection

Fig no. 23 Root end cavity

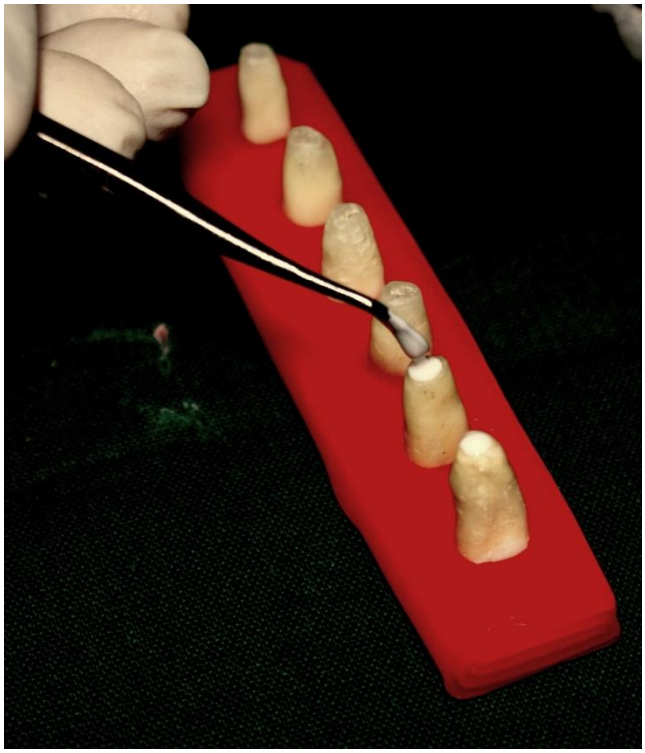


Fig no.24 Root end filling placed

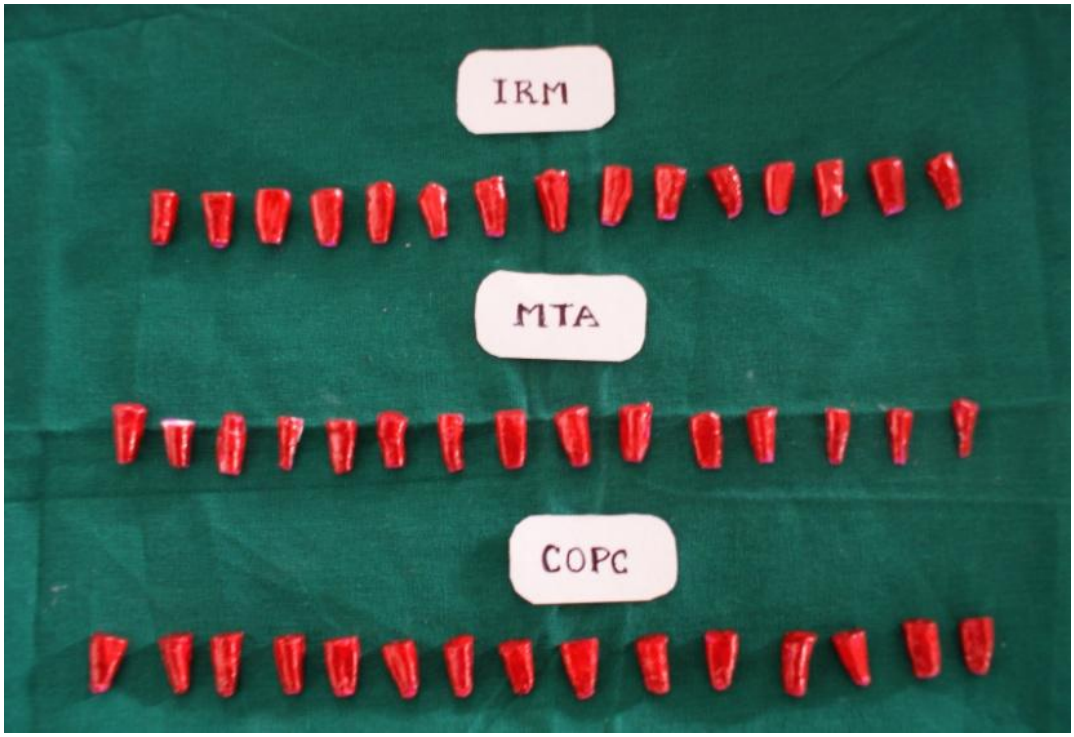


Fig no. 25 specimens coated with nail varnish

Fig no: 26 specimens stored in rhodamine B dye

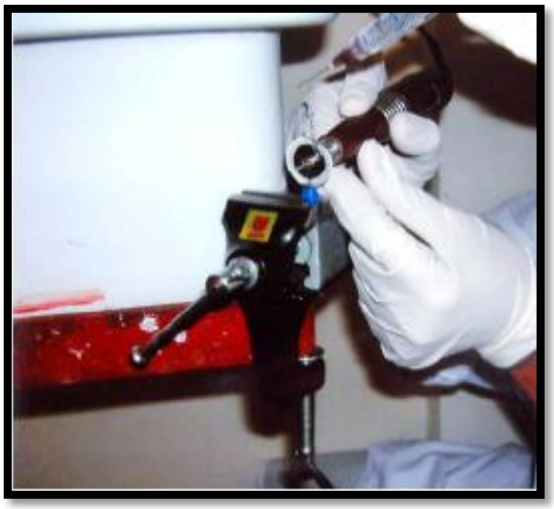


Fig no: 27 Sectioning the specimen fixed in vice

Cytotoxicity

The toxicity of castor oil polymer cement is compared and measured with commercially available materials in vitro .NIH 3T3 cell lines were obtained from Anna University, Biotechnology department and cells were grown in T^{25} culture flasks at 37 degree C in humidified atmosphere ,5% CO_2 and 95% air. A satisfactory attachment of fibroblast to the culture flasks was obtained in 24-48 hrs. The confluency of cells in the flasks was checked under an inverted phase contrast microscope.

Fibroblast subcultures were prepared from the primary culture by removing the spent medium and dissociating the fibroblast from the out growth by treatment with 2% trypsin and .2M EDTA. This method is called as trypsinization. The treated cells were incubated at 37 degree C for 3 min. The cells were dislodged from the flask by gentle tapping and later enzyme activity was stopped by adding 5 to 8mL of DMEM .The cells were subcultured by a similar method. Fibroblast used in the study was between 2 and 4 passages.

Methodology

IPP two 24 wells were selected. They were divided into 4 groups of 9 wells each.(n=9)

Group A control (without experimental material)

Group B IRM

Group C MTA

Group D COPC

Equal volumes of experimental materials was placed in each well (fig no.28).Later 1 ml of cell suspension which containing 4000 cells were seeded onto each well and incubated at 37 degree C with 5% CO_2 and 95% air.(fig no.29) At the end of 24 hrs, 48 hrs and 72 hrs they were tested for cytotoxicity using MTT assay.

MTT assay

MTT assay is a colorimetric assay that measures the reduction of 3(4,5Dimethly thiazol 2 yl) 2,3 Diphenly tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase enzyme. The MTT salt enters the cells and passes into the mitochondria where it is reduced to insoluble purple formazan product upon cleavage of the Tetrazolium by dehydrogenase enzyme. The cells were then solubilised with an organic solvent and the pink solubilised formazan reagent is measured spectrophotometrically. Reduction of MTT can occur only in metabolically active cells and the level of activity is a measure of the viability and proliferation of the cells. Results were recorded as optical density (OD) units and a decrease in OD value denotes decrease in cell viability (i.e) increase in cytotoxicity[49].

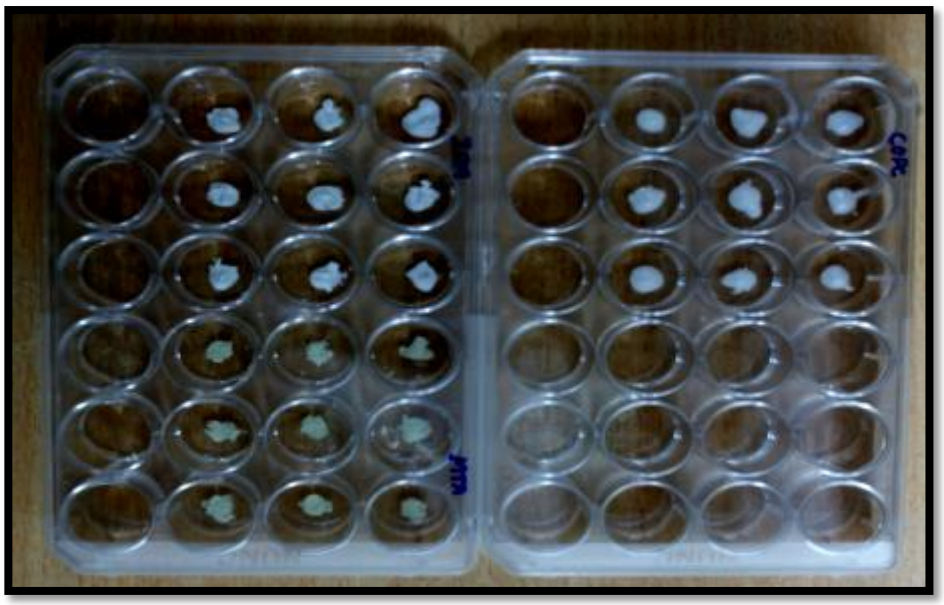


Fig no.:28 IRM ,MTA and COPC placed in 24 well

Fig no. 29 cell lines seeded in well

Procedural sequence for micro leakage

Sixty freshly extracted human maxillary central incisors selected

↓

Teeth cleaned and sectioned at CEJ using diamond disc

↓

cleaning and shaping by K files , apical enlargement done upto 50 K file size

↓

Canals obturated with GP &zinc oxide eugenol sealer by lateral condensation

↓

Access cavity is sealed with glass ionomer cement

↓

Root end resected apically at 90 ^{0}angle to the long axis of the root

↓

Group A	**Group B**	**Group C**	**Group D**
CONTROL	**IRM**	**MTA**	**COPC**

3mm deep retrograde cavity was prepared using straight fissure bur

↓

Root end filling materials placed in cavity

↓

Specimens coated with 3 coats of nail varnish except retrograde filling

↓

Specimens suspended in 2 % Rhodamine B for 24 hrs following which roots were rinsed under water for 1 hour

↓

Teeth were split longitudinally with diamond disc

↓

Depth of penetration is measured under stereomicroscope(2X) and micro leakage with different root end filling materials was evaluated in millimeters.

Procedural sequence for cytotoxicity

NIH 3T3 cell lines were grown in T^{25} culture flasks at 37 degree C in humidified atmosphere 5% CO_2 and 95% air.

↓

Attachment of fibroblast to the culture flasks was obtained in 24- 48 hrs, checked under an inverted phase contrast microscope.

↓

Fibroblast subcultures were prepared from the primary by trypsinization.

↓

The treated cells were incubated at 37 degree C for 3 min.

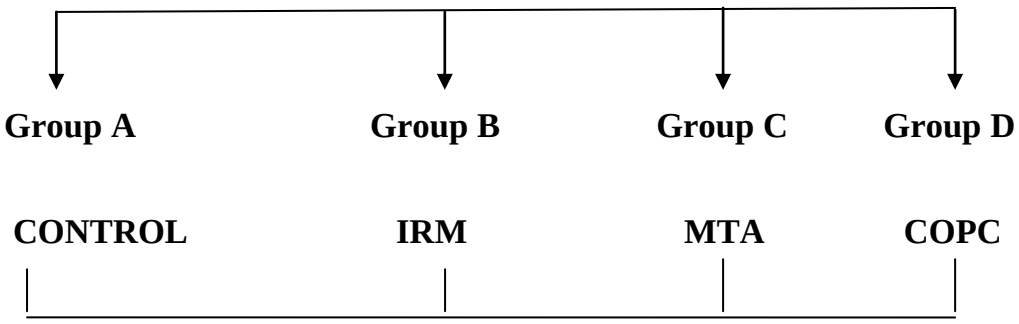
↓

Sub culturing done and cells from 2 and 4 passages were used for the study

↓

IPP two 24 wells were selected samples divided into 4 groups of 9 wells each

↓



↓

Equal volumes of material placed in each well

↓

1 ml of cell suspension containing 4000 cells are seeded onto each well and incubated

↓

Two 24 well were incubated at 37 ^{0} C for 1 hr.

↓

Centrifuge at 200 g for 5 mt

↓

Carefully aspirate the supernatant, re suspended in fresh PBS and re centrifuge

↓

Remove the supernatant and add 100 µl of isopropanol

↓

Whirl mix to solubilize the formazan product

↓

Transfer to a cuvette and read absorbance in UV spectrophotometer at 570nm

↓

Results were recorded as optical density (OD) units and a decrease in OD value denotes decrease in cell viability (i.e) increase in cytotoxicity

Results:

Statistical software SPSS 11.5 was used for analyzing the data and for generating the graphs and tables

Microleakage :

Microleakage Values (Table l)

S.NO	Group A CONTROL(mm)	Group B IRM(mm)	Group C MTA(mm)	Group D COPC(mm)
1	6	1.89	2.45	.45
2	4.32	2.2	1.8	.38
3	5.53	2.7	1.14	1.2
4	6.4	1.58	1.65	.83
5	4.2	1.78	3	.79
6	5.73	2.91	1.97	1.4
7	6.12	1.45	1.4	.73
8	4.32	1.48	1.3	.93
9	5.87	2.23	1.8	.93
10	6.23	1.90	1.24	1.12
11	5.82	2.38	1.32	.81
12	3.24	2.2	1.28	.97
13	4.78	2.4	1.13	.84
14	6.83	2.57	1.31	1.1
15	5.41	1.92	1.39	.98

The microleakage values estimated for different groups were in Table l.

Results of one way ANOVA test for leakage showed $p<.001$ as statistically significant diffrence in results within the group. (table 2)

Table 2

Descriptives

MICROLEAKAGE VALUES

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
GROUP A (CONTROL)	15	5.39	.998	.258	4.83	5.94	3	7
GROUP B (IRM)	15	2.11	.442	.114	1.86	2.35	1	3
GROUP C (MTA)	15	1.61	.528	.136	1.32	1.90	1	3
GROUP D (COPC)	15	.90	.263	.068	.75	1.04	0	1
Total	60	2.50	1.838	.237	2.03	2.98	0	7

Table 3

Multiple Comparisons

Dependent Variable: MICROLEAKAGE VALUES

Tukey HSD

(I) MATERIALS	(J) MATERIALS	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
GROUP A (CONTROL)	GROUP B (IRM)	3.281*	.227	.000	2.68	3.88
	GROUP C (MTA)	3.775*	.227	.000	3.17	4.37
	GROUP D (COPC)	4.489*	.227	.000	3.89	5.09
GROUP B (IRM)	GROUP A (CONTROL)	-3.281*	.227	.000	-3.88	-2.68
	GROUP C (MTA)	.494	.227	.141	-.11	1.09
	GROUP D (COPC)	1.209*	.227	.000	.61	1.81
GROUP C (MTA)	GROUP A (CONTROL)	-3.775*	.227	.000	-4.37	-3.17
	GROUP B (IRM)	-.494	.227	.141	-1.09	.11
	GROUP D (COPC)	.715*	.227	.013	.11	1.31
GROUP D (COPC)	GROUP A (CONTROL)	-4.489*	.227	.000	-5.09	-3.89
	GROUP B (IRM)	-1.209*	.227	.000	-1.81	-.61
	GROUP C (MTA)	-.715*	.227	.013	-1.31	-.11

*. The mean difference is significant at the .05 level.

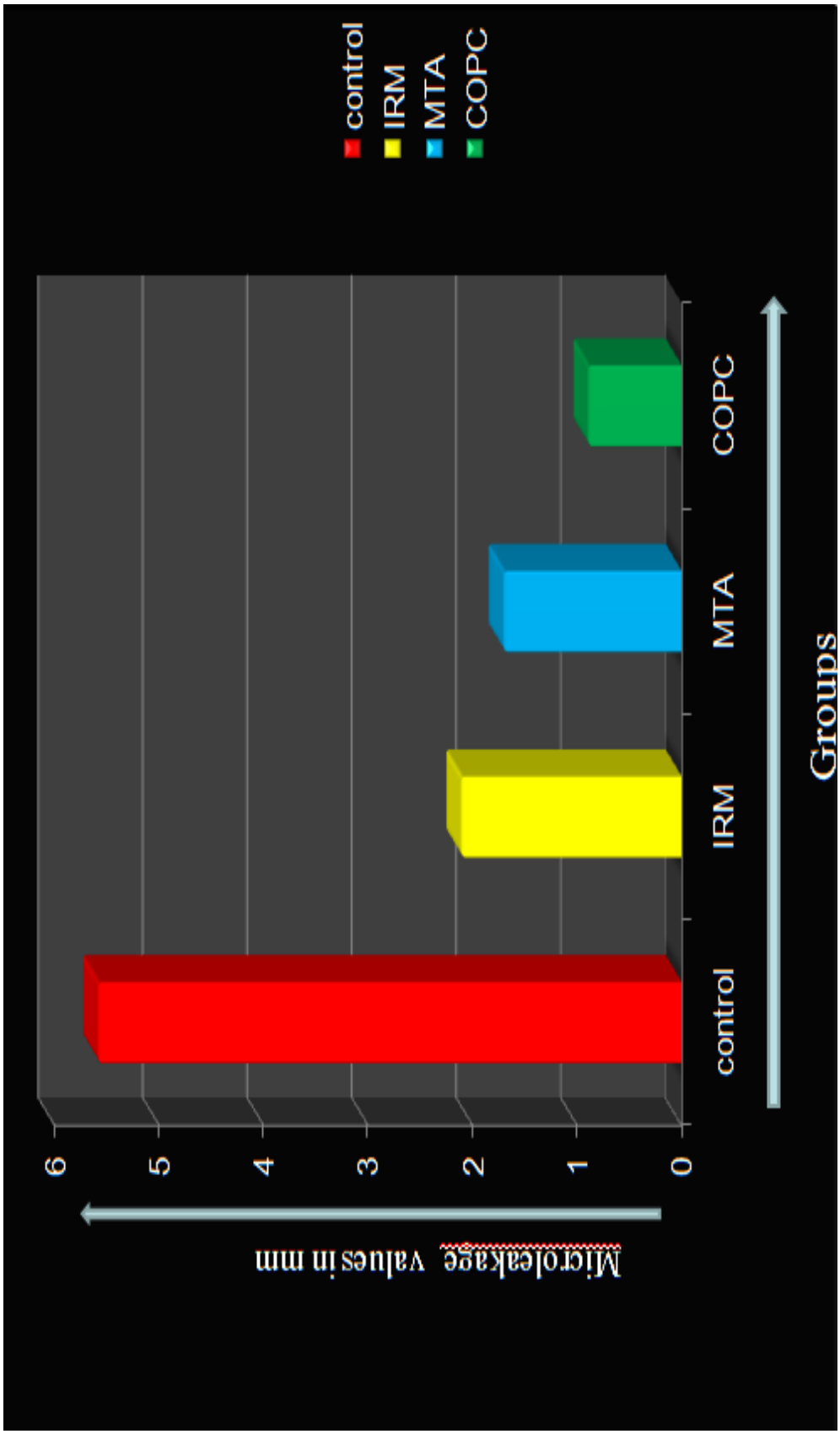


Fig no :30 Histogram representation of Microleakage

Dye penetration viewed under optical microscope (2X magnification)

Fig no.31 control(shows more microleakage)

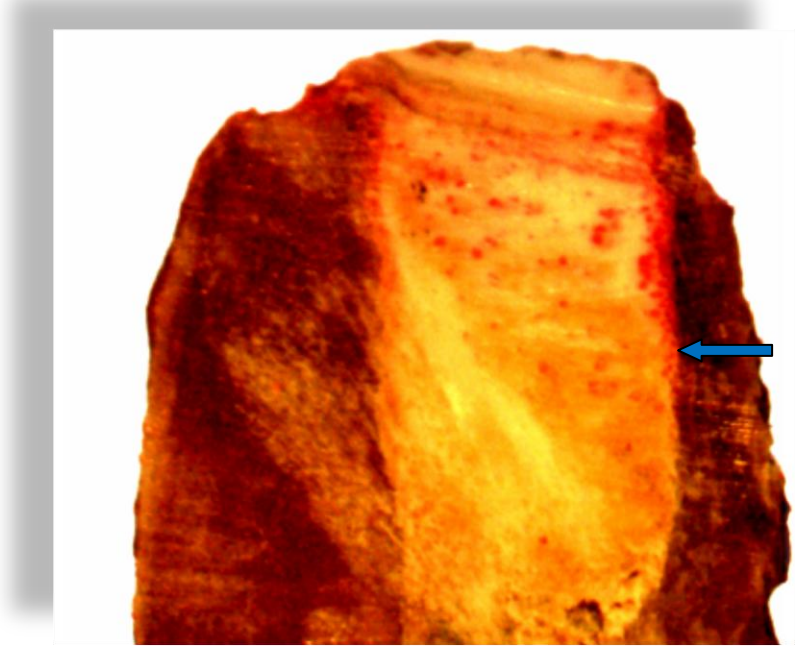


Fig no: 32 IRM(shows microleakge along tooth interface)

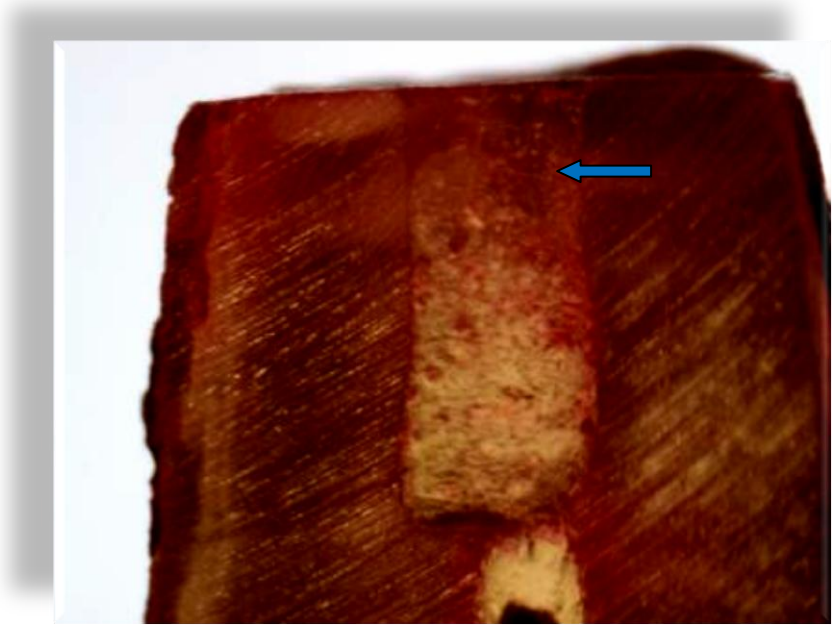


Fig no :33 MTA (shows dye penetration in to material)

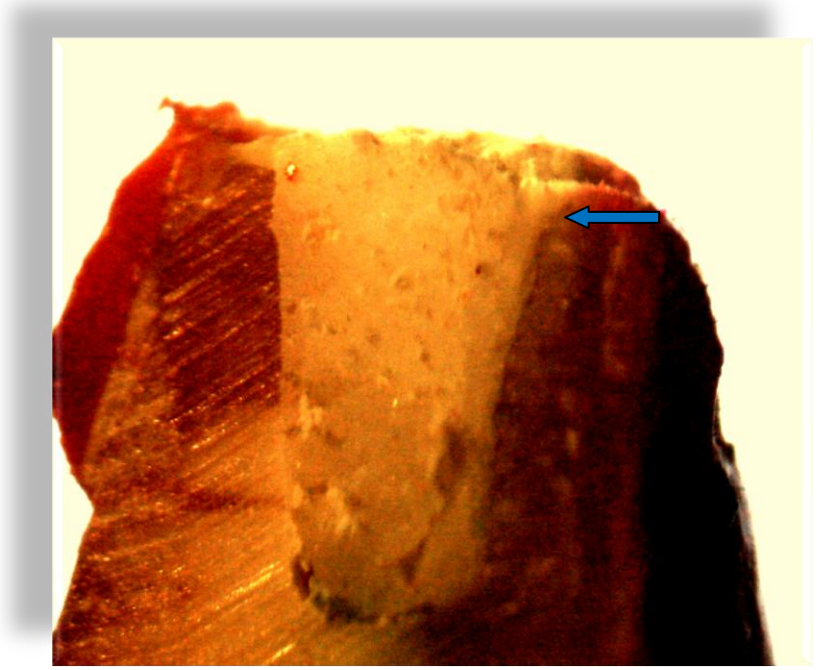


Fig no : 34 COPC(shows minimum leakage)

MTT assay.

OPTICAL DENSITY VALUES (Table 4)

MATERIALS	SAMPLES	24 HRS	48HRS	72 HRS
Group A control	**1**	**.15**	**.23**	**.34**
	2	**.14**	**.33**	**.76**
	3	**.22**	**.37**	**.88**

MATERIALS	SAMPLES	24 HRS	48HRS	72 HRS
Group B IRM	**1**	**.24**	**.43**	**.55**
	2	**.25**	**.39**	**.42**
	3	**.27**	**.36**	**.41**

MATERIALS	SAMPLES	24 HRS	48HRS	72 HRS
Group C MTA	**1**	**.37**	**1**	**1.45**
	2	**.39**	**1.80**	**1.88**
	3	**.27**	**.78**	**1.64**

MATERIALS	SAMPLES	24 HRS	48HRS	72 HRS
Group D COPC	**1**	**.19**	**.32**	**.52**
	2	**.24**	**.28**	**.76**
	3	**.23**	**.36**	**.83**

The optical density ofthe medium is evaluated using uv photospectrometer.Statistical analyses is done using Kruskal wallis test and Post hoc test .

Post Hoc Tests **Table 5**

Multiple Comparisons

Dependent Variable: 24 HRS

Tukey HSD

(I) MATERIALS	(J) MATERIALS	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
GROUP A (CONTROL)	GROUP B (IRM)	-.08333	.03408	.145	-.1925	.0258
	GROUP C (MTA)	-.17333*	.03408	.004	-.2825	-.0642
	GROUP D (COPC)	-.05000	.03408	.497	-.1591	.0591
GROUP B (IRM)	GROUP A (CONTROL)	.08333	.03408	.145	-.0258	.1925
	GROUP C (MTA)	-.09000	.03408	.110	-.1991	.0191
	GROUP D (COPC)	.03333	.03408	.765	-.0758	.1425
GROUP C (MTA)	GROUP A (CONTROL)	.17333*	.03408	.004	.0642	.2825
	GROUP B (IRM)	.09000	.03408	.110	-.0191	.1991
	GROUP D (COPC)	.12333*	.03408	.028	.0142	.2325
GROUP D (COPC)	GROUP A (CONTROL)	.05000	.03408	.497	-.0591	.1591
	GROUP B (IRM)	-.03333	.03408	.765	-.1425	.0758
	GROUP C (MTA)	-.12333*	.03408	.028	-.2325	-.0142

*. The mean difference is significant at the .05 level.

Kruskal-Wallis Test

Table 6

Test Statistics[a,b]

	24 HRS
Chi-Square	9.567
df	3
Asymp. Sig.	.023

a. Kruskal Wallis Test

b. Grouping Variable: MATERIALS

Post Hoc Tests

Table 7

Multiple Comparisons

Dependent Variable: 48 HRS

Tukey HSD

(I) MATERIALS	(J) MATERIALS	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
GROUP A (CONTROL)	GROUP B (IRM)	-.08333	.22217	.981	-.7948	.6281
	GROUP C (MTA)	-.88333*	.22217	.017	-1.5948	-.1719
	GROUP D (COPC)	-.01000	.22217	1.000	-.7215	.7015
GROUP B (IRM)	GROUP A (CONTROL)	.08333	.22217	.981	-.6281	.7948
	GROUP C (MTA)	-.80000*	.22217	.029	-1.5115	-.0885
	GROUP D (COPC)	.07333	.22217	.987	-.6381	.7848
GROUP C (MTA)	GROUP A (CONTROL)	.88333*	.22217	.017	.1719	1.5948
	GROUP B (IRM)	.80000*	.22217	.029	.0885	1.5115
	GROUP D (COPC)	.87333*	.22217	.018	.1619	1.5848
GROUP D (COPC)	GROUP A (CONTROL)	.01000	.22217	1.000	-.7015	.7215
	GROUP B (IRM)	-.07333	.22217	.987	-.7848	.6381
	GROUP C (MTA)	-.87333*	.22217	.018	-1.5848	-.1619

*. The mean difference is significant at the .05 level.

Kruskal-Wallis Test

Table 8

Test Statistics[a,b]

	48 HRS
Chi-Square	8.453
df	3
Asymp. Sig.	.038

a. Kruskal Wallis Test

b. Grouping Variable: MATERIALS

Post Hoc Tests Table 9

Multiple Comparisons

Dependent Variable: 72 HRS

Tukey HSD

(I) MATERIALS	(J) MATERIALS	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
GROUP A (CONTROL)	GROUP B (IRM)	.20000	.16298	.628	-.3219	.7219
	GROUP C (MTA)	-.99667*	.16298	.001	-1.5186	-.4748
	GROUP D (COPC)	-.04333	.16298	.993	-.5652	.4786
GROUP B (IRM)	GROUP A (CONTROL)	-.20000	.16298	.628	-.7219	.3219
	GROUP C (MTA)	-1.19667*	.16298	.000	-1.7186	-.6748
	GROUP D (COPC)	-.24333	.16298	.484	-.7652	.2786
GROUP C (MTA)	GROUP A (CONTROL)	.99667*	.16298	.001	.4748	1.5186
	GROUP B (IRM)	1.19667*	.16298	.000	.6748	1.7186
	GROUP D (COPC)	.95333*	.16298	.002	.4314	1.4752
GROUP D (COPC)	GROUP A (CONTROL)	.04333	.16298	.993	-.4786	.5652
	GROUP B (IRM)	.24333	.16298	.484	-.2786	.7652
	GROUP C (MTA)	-.95333*	.16298	.002	-1.4752	-.4314

*. The mean difference is significant at the .05 level.

Kruskal-Wallis Test

Table 10

Test Statistics[a,b]

	72 HRS
Chi-Square	7.269
df	3
Asymp. Sig.	.064

a. Kruskal Wallis Test

b. Grouping Variable: MATERIALS

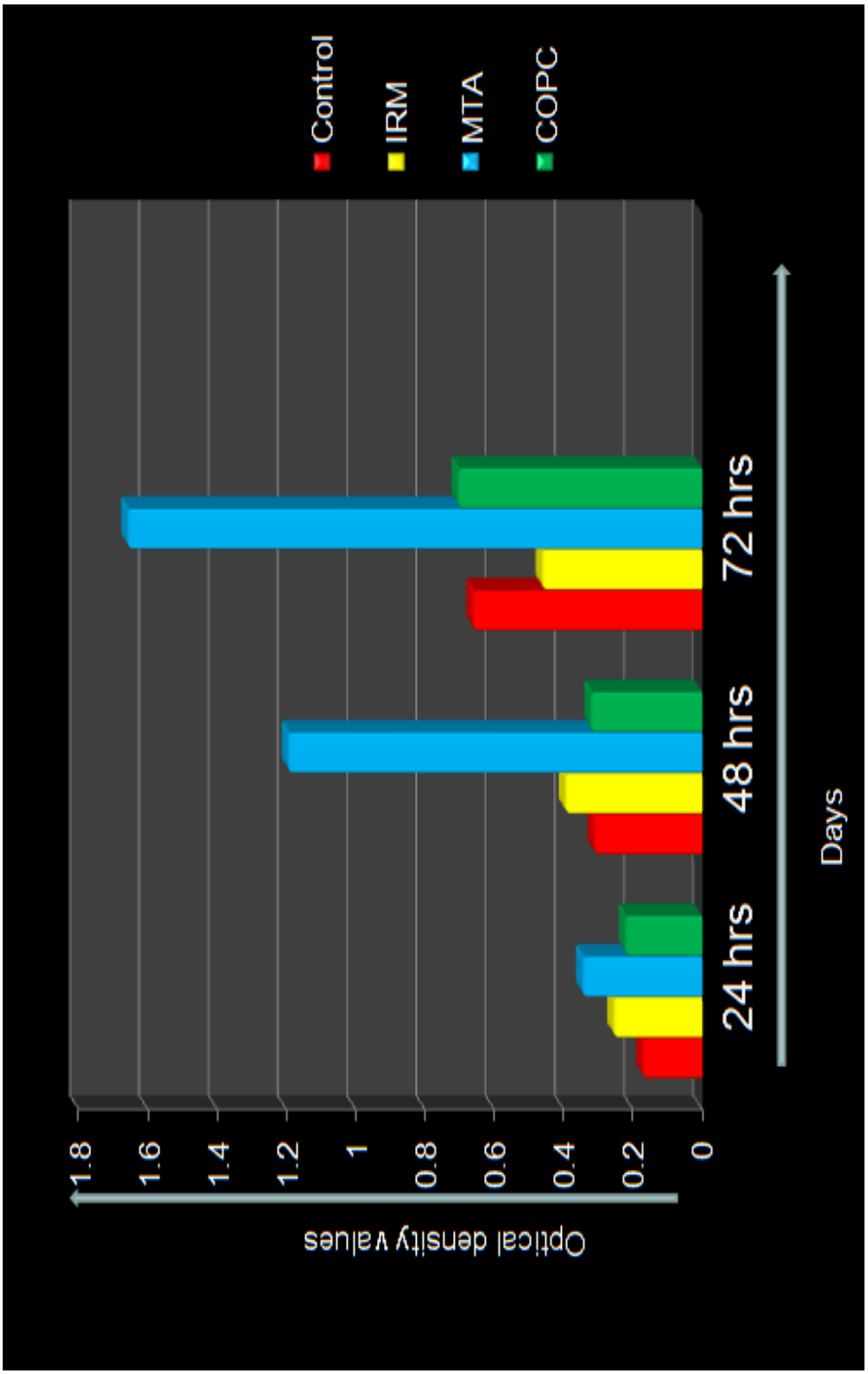


Fig no :35 Histogram representation of optical density

In Kruskal Wallis test(tab 6 ,8and 10) , COPC shows significant difference in 24 hrs and 48hrs In 72 hrs($p>.05$)there were no significant difference between group B(IRM) Group A(control)and group D(COPC), which shows that COPC shows marked cell growth like in all other groups . In Post hoc test (multiple comparison) between groups(tab 5,7and 9),group C shows significant difference among the other groups ($p<.05$)which shows MTA promotes more cell growth than COPC and IRM.

Cell growth viewed under phase contrast microscope (X10 magnification)

Cell growth after 24 hrs

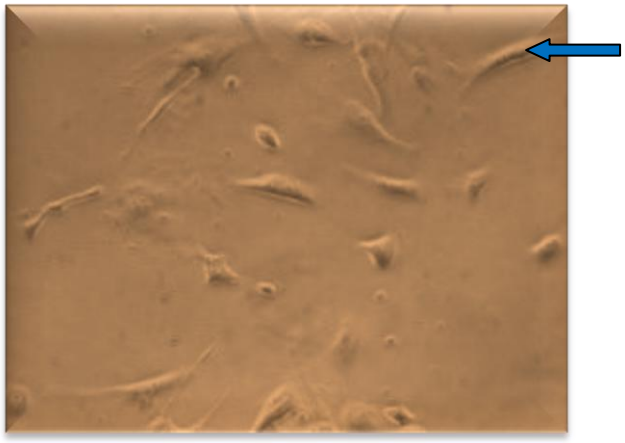


Fig no:36

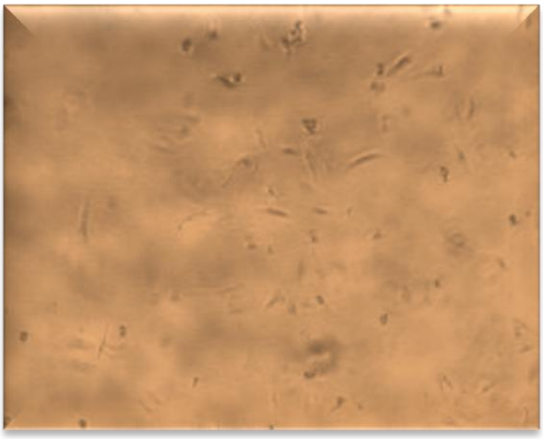


Fig no:37 IRM

Control (confluent spindle shaped cells)

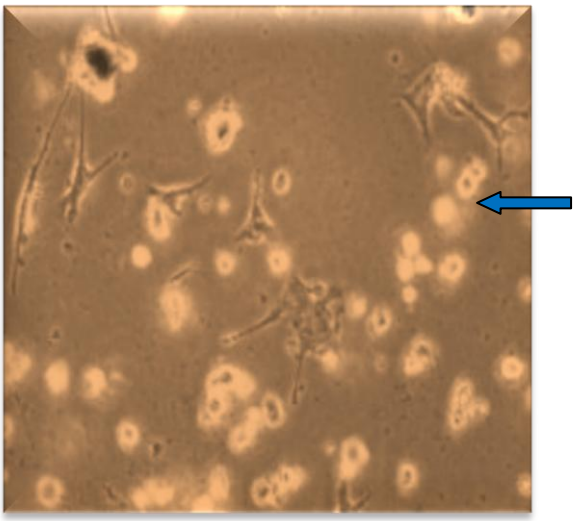


Fig no:38 (confluent cell growth)

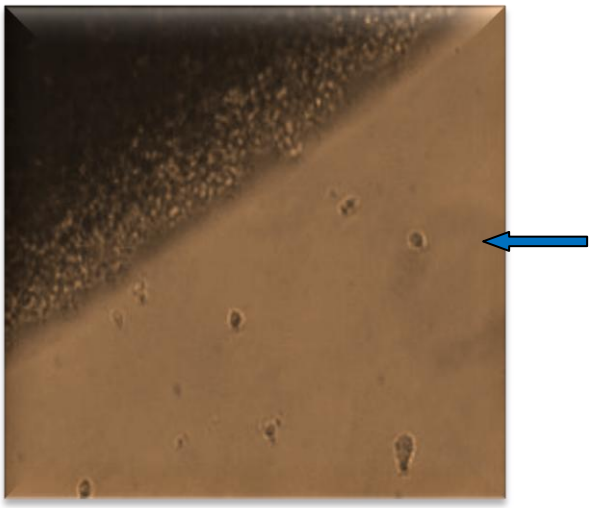


Fig no:39 COPC (round shaped cells present)

Cell growth after 48 hrs

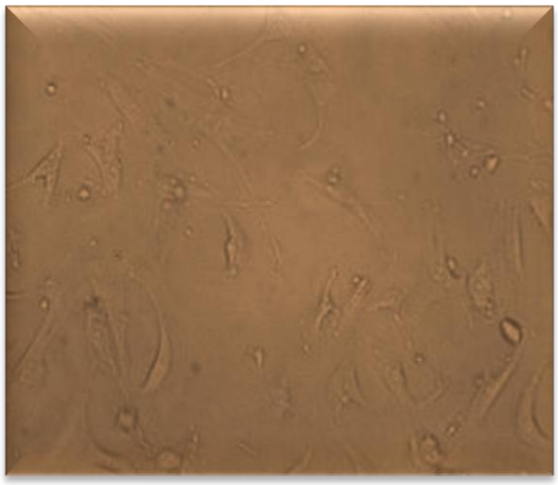


Fig no:40 control

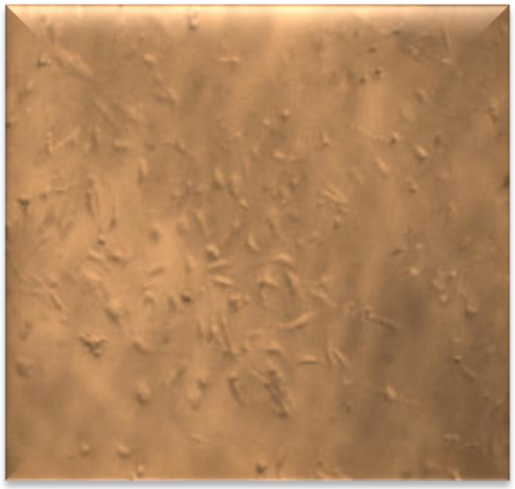


Fig no:41 IRM

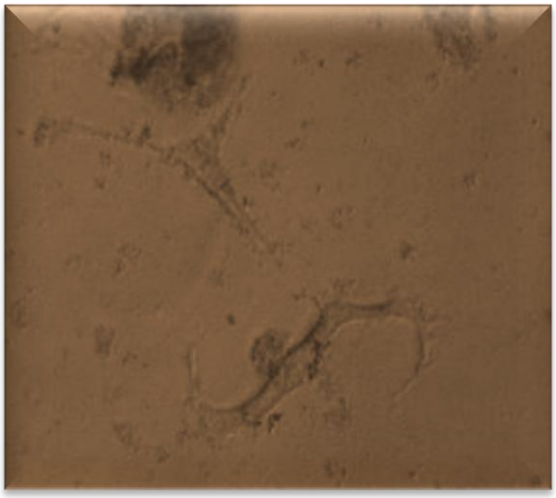


Fig no:42 MTA(spindle shaped cell)

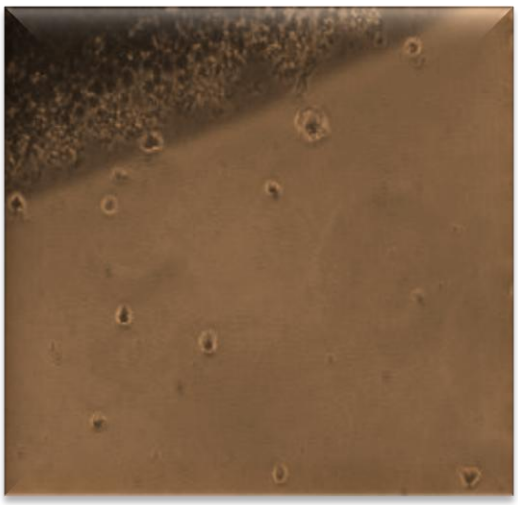


Fig no:43 COPC(round shaped cells)

Cell growth after 72 hrs

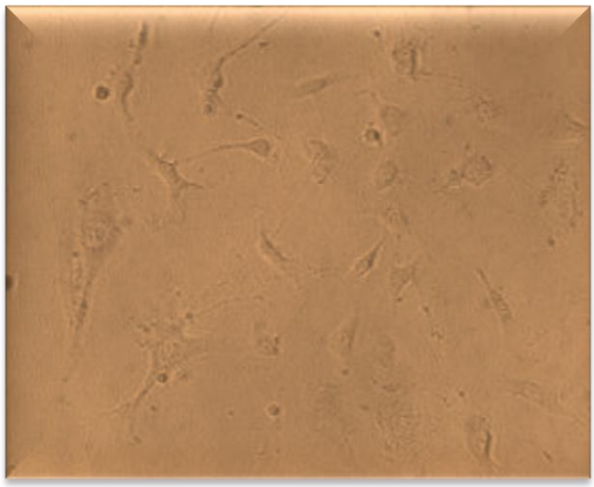


Fig no:44 Control

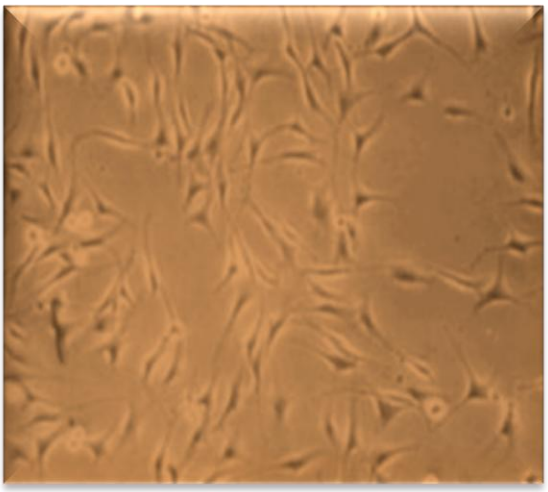


Fig no:45 IRM

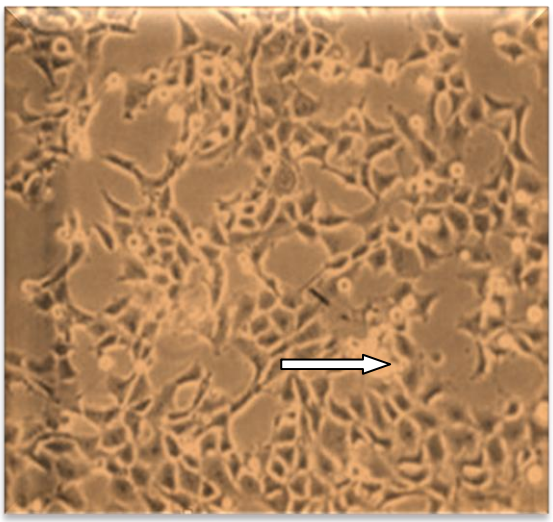


Fig no:46 MTA shows cluster of dead cells

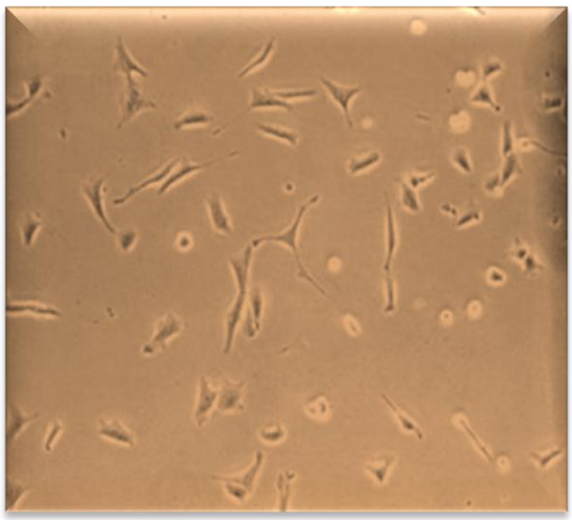


Fig no: 47 COPC (confluent cell growth)

Discussion

Apical surgery is performed in the presence of persistent periradicular pathosis when orthograde endodontic treatment is unfeasible . Some endodontic failures are due to inadequate cleaning of root canals and egress of antigens into periradicular tissues . A number of investigators have recommended placement of root end filling in teeth that require root end resection[36].

The study to compare micro leakage with various root end filling materials can be carried out by both in vivo and in vitro methods. But due to limitations with in vivo studies like large number of specimens, time consumption, and patient follow up, in vitro study was done.

Various methods have been employed to assess the micro leakage of root end filling materials like degree of dye penetration , radio isotope penetration , bacterial penetration and electrochemical means and fluid penetration technique .Dye penetration , bacterial leakage and fluid penetration are some methodological approaches used to evaluate apical sealing ;however contradictory findings are still observed in the literature.[6,24,70,79,80.] Each technique has significant limitations that can result in errors. The most frequently used technique is linear measurement of dye penetration.

This study deals with semi quantitative analyses of linear dye penetration with disadvantages often involving only one plane of view, but it is the preliminary method to evaluate the marginal leakage of new materials.

According the Aqrabawi et al [4] dye penetration tests are easy and useful method to evaluate root end filling materials because, if the materials are able to prevent the leakage

of small molecules (tracer solutions), they should prevent the infiltration of bigger substances such as bacteria and their by products.

This method of dye penetration allows for the production of sections showing leakage in contrasting colors to both tooth and restoration without the need for further chemical reaction.

Rhodamine B dye was chosen for this study because of high degree of penetration into apical dentin .According to Azoubel, Reeck[5], Rhodamine B could be applied in studies of dye penetration because it has smaller particles, presenting greater diffusibility in dentinal tubules and because it is easily evaluated .Rhodamine B dye presented more penetrability in apical dentin and such findings could be related to the greater facility of visualization when compared to methylene blue [30.]

Hamaoka, Mousa [31] and Souza [64] got corroboratic results when comparing several tracer solutions in the apical region, they observed that Rhodamine B demonstrated higher leakage. According to Tanoman Filho et al [66,] Rhodamine B is the most appropriate tracer solution to evaluate sealing capability of MTA; however it has been used in a few studies when compared to other dye solutions.

A controversial topic in the literature is the moment at which the specimens should be immersed in the dye solution[55]. In the present study immediate immersion in the dye by passive dye penetration technique at 37 ^{o}C and 100% humidity for 24 hrs was selected based on the fact that in the clinical situation the root end material will be in contact with secretions like blood, soon after the insertion in the cavities.

The inclined plane sectioning at 30 or 45 degree could have disadvantage like open dentinal tubules, errors in post operative radiographs, more mechanical stress, and loss of dentin, cementum and bone that could result in compromised healing . Plane of sectioning also affects the degree of microleakage, so the root canal resection angle of 90 degree was selected for this study[58]

Apical ramifications and lateral canals are very common near the apical end and the preferred root resection is 3mm [58.]Resection at the depth of 3 mm reduces the apical ramification by 98% and lateral canals by 93%.In this study resection of root was performed at the depth of 3mm to eliminate any lateral canals or apical ramification .

For making cavity preparations in root end we can use various instruments like conventional slow speed , high speed air rotor hand pieces with burs ,Sonics and ultrasonics . According to previous studies the ultrasonic root end preparation produces more conservative cavities when compared to conventional micro motor hand piece bur [44]. But we chose to prepare root end cavities using conventional micro motor hand pieces and burs as in contrast to some studies have also shown that root ends prepared by ultrasonics had a significantly greater number of fractures. [48]

The depth of penetration ideally should be 3 mm as more than that does not bestow any greater benefits whereas lesser depth may jeopardize the long term success of apical seal .Increasing the depth to an optimum decreases the leakage [28.]This is attributed to the occlusion of apical tubules by retro filling material. Hence the depth of retrograde cavities in this study was kept to an optimum of 3mm.

The choice of retro filling material should be governed by biocompatibility, apical sealability, handling properties and long term success [37].Most in vitro studies evaluate leakage of apical seals, but the correlation between dye leakage around retro filling materials and their clinical performance is uncertain[25.]The clinical significance of micro leakage in apical surgery has not been elucidated. However it seems logical that the lesser leakage would prevent migration of bacteria and tissues in to the periradicular tissues [37.]

It was decided to use IRM as comparative material because it has been widely used and gold standard material for root end filling, since amalgam seems to have higher toxicity to human periodontal ligament cells and human osteoblast like cells than IRM [82].

MTA is another promising root end filling material that has received much attention recently. MTA has shown to have less leakage than amalgam or zinc oxide eugenol materials in leakage test.[69,73] Other properties that have been investigated include its in vitro cytotoxicity and biocompatibility when embedded in bone and subcutaneous connective tissue. [72,81]

In this study IRM and MTA were selected to compare sealing ability of Castor Oil Polymer Cement (COPC) as root end filling . COPC is prepared from NCO terminated pre polymer and castor oil and calcium carbonate . The chemical composition of this material presents a chain of fatty acids whose molecular structures are also present in lipid of human body.

Castor Oil Polymer Cement (COPC) is prepared making NCO terminated pre polymer to react with castor oil which has numerous hydroxyl groups that add more benefit in biocompatibility . The derivation of hydroxyl ions from the castor oil is eliminated

in the Castor Oil Polymer Cement (COPC) which makes difference in preparation from commercially available COP.

All these properties favour its use as root end filling material ,hence COPC was experimented to compare with existing material MTA and IRM .

The statistical analyses showed that all materials have significant microleakge of dye when compared with control group(without root end filling) . When COPC is compared with IRM and MTA ,COPC (1.12mm) shows significantly less liner dye penetration than MTA(2.45mm) and IRM (2.91mm) .This result was congruent with commercially available COP which also shows less leakage than MTA.

The results from the previous dye penetration studies done by Torabinejad [74],Martell [46] and Pereria[51] showed that MTA exhibits significantly less marginal leakage when compared with other root end filling materials like IRM ,Super EBA and amalgam. In apical leakage of protein dye complex also showed that 4 mm thickeness of MTA is more effective in preventing microlekage.[75] The clinical study done by Chong et al [15] and Spanberg[65] showed controversial results for MTA . They found that there were no significant difference in success rate of MTA and IRM

In this study also ,there were contradicatory results exhibited ,that there were no significant difference between MTA and IRM. This may be due to the penetration of dye more into the material than into the tooth interface which is congruent to the study done by Camilleri et al [13]. He reported that MTA was less affected by changes in dye pH, but there was an increased susceptibility of leakage within the material rather than at the tooth to material interface.

Although MTA has superior biocompatibility when compared to the traditional materials used in root end filling and root repair , material is costly and has poor handling characteristics . Despite the numerous carrier devices available in the market to help in its clinical placement , clinicians are still finding MTA difficult to use in certain surgical situations because of the location of the surgical site and small size of the root end preparation[43].

Some studies with novel materials were done to overcome the handling properties and cost effective benefits . Lysanda paste (zinc oxide eugenol paste)with radio opacifiers exhibits lower leakage without statistically significant difference when compared with MTA[10]. Viscocity Enhanced Root repair material (VERRM) showed physical properties and sealing ability similar to WMTA .[34]

In previous study done by Martins , it showed that castor oil polymer (COP) had less linear dye penetration when compared with MTA in prepared cavity depth of about 1.25 mm [47] .The results in this study COPC(1.12mm) also exhibits congruent results with COP . The linear dye penetration of dye for MTA and IRM is more than 1.25mm , from which it is assumed that 1.25 mm depth of cavity preparation for COPC may also give better apical seal like COP.The minimum dye penetration in COPC could be due to the adhesive properties of isocyanate . Further studies and research are required in the field of adhesion to the tooth structure .

According to the results presented in microleakage evaluation of COPC, it can be considered a potential material for sealing root end cavities and may be suitable for

clinical use .However , the biocompatibility of COPC needs to be assessed and compared with IRM and MTA to further validate its consideration for clinical use .

In this study IRM and MTA were selected to compare the cytotoxic effects as these materials are in close contact with live tissue [18] . The toxic effects of materials used for endodontic therapy are of particular concern, because damage or irritation could cause degeneration of the peri-apical tissue and delayed healing. *In vivo* test such as implantation and usage test have an advantage in that they allow complex interaction between the host and the material to be examined. *In vitro* test such as cell cultures enable experimental factors and variables to be controlled which often is a significant problem when performing experiments in vivo. These *in vitro* model assays are increasingly being used for initial screening for new dental materials intended for clinical use.

A variety of test systems are available to determine the cytotoxicity of dental materials in cultured mammalian cell populations. Permeability assays monitor the integrity of cell membranes by the inclusion or exclusion of vital dyes or by the release of radio-labeled chromium. Replication assays indirectly assess the ability of cells to proliferate by measuring the incorporation of nucleotide analogues that have been radio-labeled or are detectable by immunoassay during DNA synthesis. Changes in the cellular cytoskeleton or at the cell surface are observed by morphological studies. Finally, functional assays typically evaluate the cell's ability to provide the energy necessary for anabolic activities or the end products of such activities.

The assay used in the present study used the tetrazolium salt MTT to measure mitochondrial dehydrogenase activity. It is a yellow substrate that produces a dark blue formazan product when cleaved by active mitochondria. Therefore, the reaction only occurs in living metabolically active cells. The decision to use a particular test system should be based on its consonance with the chemical nature of the material being tested. For example, if a material is not likely to cause a change in the permeability of cell membranes, a permeability assay is less apt to determine cytotoxicity in a valid manner. Because MTA is a hydrophilic substance, it is likely to release ionic components. It would be more apt to interfere with intracellular enzyme activities than influence membrane permeabilities [18]. Therefore, the MTT assay was chosen in the present study.

It is critical to select the appropriate cell types for cytotoxicity assays. International standard prefers the use of established cell lines such as L 929 ,Blab/ NIH 3T3 and WI 38 for cytotoxicity testing. These cell lines provide good reproducibility for in vitro cytotoxicity screening owing to their homogenous morphology and growth characteristics[39]. On experimental basis NIH 3T3 mouse fibroblast was selected for this study.

Freshly mixed materials are placed in the wells, as they release materials during chemical reactions which may have more cytotoxicity. Most of the studies were based on either quantitative or qualitative assessment. In this study both qualitative assessment including morphologic evaluation applying optical microscopy and quantitative assessment with cell functional tests were accomplished. Thus according to quality and quantity

assessment in this investigation , it possesses the privilege that what was observed in optical microscope qualitatively was also evaluated quantitatively using MTT assay test.

Finding of this study presented that COPC, IRM and MTA do not induce cytotoxicity on NIH 3T3 in MTT assay. A confluent cell culture was observed in the control group maintained for the whole time of the experiment .

In first 24 hrs , on optical miocroscopy visualisation c,onfluent cell were present in MTA and there were no morphological differentiation in IRM and COPC(fig no.3 39) .But the optical density values shows cell growth when compared with other groups. This variation in results may be due to two factors: cell attachment and cell growth. Cell attachment belongs to the first phase of cell/material interactions and the quality of this phase will influence the cell capacity to proliferate and to differentiate itself on contact with the material.[3,19.]

Factors such as the hydrophilicity/ hydrophobicity[78] and surface energy[16] and charge[78,79] of the material greatly influence the cell attachment and growth. In addition, surface roughness would be enhanced by adsorption of the proteins in the culture medium to form biofilms[11] which could mediate the cell adhesion. The above mentioned factors may influence in attaining confluent cell growth . Further research is needed to evaluate the physical and biological properties of COPC.

At 48 hrs , COPC shows round shaped cells and morphological differentiation of cells were not found .But the optical density values were increased when compared with 24 hrs . (fig no.40-43)

At 72 hrs ,COPC attains spindle shaped cells and the marked cell growth was present in all the groups. MTA shows increased cell growth than other groups .At this stage ,MTA shows marked cell growth and cluster of dead cells were noticed when compared with other groups.(fig no. 44 47)

On statistical analyses , there is no significant difference among COPC ,IRM and control . MTA shows statistically significant difference from other groups- COPC,IRM and Control, which shows MTA promotes marked cell growth when compared to other groups. MTA and IRM shows cell growth , which is congruent with results of previously done studies using L929 cell lines[33,35.] .

Within the limitation of this study,COPC had better sealing ability than MTA and IRM and does not induce cytotoxicity on NIH 3T3 fibroblast in both techniques including optical microscopy and MTT assay hence it can be also considered a good option as root end filling material.

Summary

The purpose of this study was to evaluate and to compare microleakage and cytotoxicity of castor oil polymer cement with IRM and MTA when used as root end filling materials. Leakage assessed using a stereomicroscope study with Rhodamine B dye as the medium

Sixty freshly extracted central incisors were instrumented and obturated with gutta-percha using lateral condensation technique. The teeth were apically resected at an angle of 90^{0} to the long axis of the root and root end cavities were prepared. The teeth were divided into four groups of fifteen specimens each and were filled with the following materials .

Group A control (without root end filling material)

Group B IRM

Group C MTA

Group D COPC

The teeth were coated with nail varnish and after drying the specimens were immersed in 2% Rhodamine B dye for 24 hrs . The teeth were rinsed for 1 hour and sectioned longitudinally .The specimens were observed under stereomicroscope and the depth of penetration was determined in millimeters. The results were analysed statistically . There was significantly less leakage exhibited with COPC when compared to IRM and MTA.

After microleakage assessment COPC is subjected to in vitro cytotoxicity evaluation using NIH 3T3 mouse fibroblast. NIH 3T3 mouse fibroblast is subcultured and cells 2^{nd} and 4^{th} passages were used for the study. IPP two 24 wells were selected - samples were divided into 4 groups of 9 wells each .(n=9)

Group A control (without experimental material)

Group B IRM

Group C MTA

Group D COPC

The test materials were placed in each well .Then the cell suspension containing 4000 cells were seeded onto each well and incubated. They were subjected to toxicity using MTT assay after 24hrs ,48 hrs and 72 hrs. The results were analysed statistically . In multiple comparison of groups ,Group C (MTA) showed marked cell growth and significant difference were present when compared to Group D (COPC) and Group C(IRM). There were no significant difference among Group D(COPC) ,GroupC(IRM) and Group A (control).The results from MTT assay concluded that COPC does not induce cytotoxicity similar to MTA and IRM .

Conclusion

The results of this study showed that COPC had better sealing ability than IRM and MTA as root end fillings and no cytotoxicity effects were found in MTT assay. Research in using COPC are limited, but definitely promising results encourage further investigations , especially on its biological properties , to confirm its qualities as root end filling material in the future.

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